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Heme Nitric Oxide and/or Oxygen (H-NOX) binding domains are gas-sensing domains found in both eukaryotic and prokaryotic cells, and are involved in vital functions such as chemotaxis and signal transduction. The heme-binding pocket of the H-NOX protein from *Thermoanaerobacter tencongensis (Tt)* has been shown to bind diatomic molecules such as O_2, NO, and CO and the protein has been proposed to be able to sense O_2 in its native environment. Crystal structures of *Tt* H-NOX have provided static structural images of the protein that suggest the heme pocket is inaccessible to solvent. Our current study focuses on probing the nature of the local hydration state of the heme pocket in addition to other sites in the protein by genetically incorporating the spectroscopic reporter unnatural amino acid (UAA) *p*-cyano-L-phenylalanine (pCNF) in a site-specific manner. pCNF is an effective vibrational reporter of local protein environments due to the sensitivity of the nitrile symmetric stretching frequency of this UAA to local environment. The hydration status of the heme pocket and other sites in the protein will be presented as determined utilizing the vibrational reporter UAA pCNF.

1873-Pos Board B17
Structural Insight into Split Green Fluorescent Protein
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Split green fluorescent proteins (GFP) with a structural element removed have potentially useful applications in the control of light-driven protein-protein interactions, and in the preparation of semi-synthetic proteins with novel spectroscopic or functional properties. The truncated GFP generated by removing the 11th β -strand has been previously shown to reassemble with synthetic strand 11 peptide only following light activation (Kent and Boxer, JACS 2011). The light irradiation is thought to drive a photo-isomerization reaction within the chromophore of the protein, switching the truncated protein from the thermally stable *trans*-like state to the strand-receptive *cis*-like state. However, neither the process by which this occurs nor the presumed conformational changes that accompany it are understood to any level of structural detail. We utilize the unique reassembly behavior of split GFP in conjunction with solution state NMR to gain some insight into the structure and dynamics of truncated GFP. The rates of hydrogen-deuterium exchange (HDX) of the amide protons in the truncated GFP compared to the rates in an intact GFP (Huang, *et al.* and Jackson, JMB 2007) serve as a proxy for solvent exposure and protein stability. The HDX experiments show evidence of residual structure in the truncated GFP, based on assignments that correspond to peaks in intact GFP. Obtaining further structural information will be instrumental in solidifying our understanding of the observed phenomena, and perhaps lead to the rational design and engineering of other split fluorescent protein systems with interesting photo-reactive behaviors.

1874-Pos Board B18
Structural and Dynamical Aspects of Electrostatic Interactions by Applying Aspherical Atom Model in HIV-1 Protease
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Accurate approximation of electrostatic energies is of major importance for our understanding protein energetics in computer-aided drug design as well as for the design of novel biocatalysts and protein therapeutics. In this study, we applied new method, based on an aspherical atom model build from University at Buffalo Database (UBDB), for estimating electrostatic interactions in HIV protease (HIV-PR). The method is much more accurate than classically used force fields. HIV-PR, an aspartyl protease enzyme involved in human immunodeficiency virus (HIV) replication, is an important target for drug design strategies to combat acquired immune deficiency syndrome (AIDS). Most of the currently used HIV-PR inhibitors in HIV treatment have been prone to suffer from the mutations associated with drug resistance. Therefore, it is necessary to search for potent alternatives helping to overcome the resistance. The active site of PR protein is formed by the dimerization of the two monomers and covered by two glycine rich, antiparallel beta-hairpins flaps. In recent study, it has been shown that monomeric HIV-PR is relatively stable as compared to its dimeric form. It is also reported that mutations at or near the monomer-monomer interface shift the monomer-dimer equilibrium to inactive monomeric form, which is now of much interest in the contexts of drug design. Such observation can be confirmed by applying molecular dynamics simulation providing ample insights into both biological and technical aspect of macromolecular conformation and dynamics at atomistic level. So it's worth to investigate the multi-conformers of HIV-PR existing due to various mutations by combining MD simulation with the UBDB derived electron density for high resolution structures ≈0.8-1.00 Å. The research may lead to the discovery of a general rule of conserved electrostatic interaction that stabilizes the HIV-PR dimer.

1875-Pos Board B19
Probing the Elastic Properties of Alpha Helices via Buckling Simulations
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Alpha helices are ubiquitous structural motifs in proteins. Moreover, their reaction to mechanical stresses is key to understanding SNARE-mediated bilayer fusion and other fundamental biological processes. Previous investigations of alpha-helical rigidity have assumed the alpha helix to be an Euler rod. In this project, we use coarse-grained buckling simulations of alpha-helical segments to measure the bending modulus k; in addition, we use shape-fitting to determine the nonlinear corrections to the Euler model.

1876-Pos Board B20
Heterotrimeric G-Protein Alpha (α) Subunit from a. Thaliana Forms Trimeric Structures in Solution
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The heterotrimeric guanine nucleotide-binding proteins (G-proteins) mediate transmission of signals from G protein coupled receptors (GPCR) to effector systems including ion channels, enzymes and intracellular second messengers in yeast, mammals and plants. The complex is comprised of alpha (Gα), beta (Gβ) and gamma (Gγ) subunits; Gα has GTP binding and hydrolysis activity, and Gβ and Gγ interact with downstream effectors as a dimeric complex. Although the structure and activation mechanism for the mammalian complex are well known, these are still not fully understood in plants. We investigate biochemical and structural features of heterotrimeric G –proteins from *A.thaliana* to gain insight into its activation mechanism and to develop a better understanding of molecular interactions in the G-protein related signaling pathways in plants[1].
Here we present the results of biochemical and structural characterization studies on the wild type AtGPA1 and an N-terminal 36-aa residue truncated version, GPA1t, produced in yeast and bacteria expression systems, respectively. Nucleotide (GTP, GDP, GTPγS) binding is confirmed by absorbance spectroscopy and Circular Dichroism Spectropolarimetry (CD) results indicate that the secondary structure of AtGPA1-GTPγS is more stable than that of AtGPA1-GDP. Dynamic light scattering (DLS) and native-Page analyses combined with small angle X-ray scattering (SAXS) measurements reveal that AtGPA1 has a tendency to form trimers in solution. SAXS data also shows that AtGPA1-GDP has a globular structure with some flexibility. The physiological significance of the trimeric structure and models calculated using SAXS data will be presented. Results will be discussed together with the crystal structure of truncated GPA1[2], on which the current proposed models of G-protein activation mechanism in plants are based.
[1] B. Kaplan (2009), PhD Thesis Sabanci University.
[2] J. C. Jones et al.(2011) *Sci. Signal.*, vol. 4, p. ra8.

1877-Pos Board B21
Protonation and Deprotonation Reaction of Aspartic Acid Side Chain Modulated by the Surrounding Dielectric Medium - AB Initio Quantum Chemical Studies on Aspartic Acid in Sixteen Different Solvents and Two Protein Structures
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Side chain of aspartic acid constitutes of carboxylate group, pKa equals to 3.7, in aqueous solution. However, large pKa shifts are often observed in protein structures. Several aspartic acid side chains are found in protonated form in protein NMR structures, for example, Asp26 in human thioredoxin, Asp96 in bacteriorhodopsin etc. Local micro-environment plays a crucial role in pKa shift of this amino acid side chain. Question asked in this study - how variation in the local dielectric medium influence protonation of aspartic acid side chain? To answer this question, we have performed Density Functional Theory (DFT) and Möller-Plesset second order perturbation theory (MP2) calculations on aspartic acid side chain in 16 different implicit solvents with varying dielectric constants. These calculations show that bond order of carboxylic-OH group decreases steeply in low dielectric range (ε between 1 to 9). Change in bond order within high dielectric range ($\varepsilon > 20$) is small. This calculation suggests that carboxylic-OH bond order in aspartic acid side chain should be high in protein hydrophobic region (low dielectric medium) compared to protein hydrophilic region (high dielectric medium).